THE RELATIVE IMPORTANCE OF STREPTOCOCCI AND LEUCOCYTES IN MILK.

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IN the general crusade for the public weal that has been carried on in this country for the last decade, much has been accomplished in providing for a more wholesome milk-supply in several of the great centers of population. Of especial interest in this connection are the records of the Department of Health of the City of New York[1] which show what has been accomplished in that place, and demonstrate what can be done elsewhere if apathy, ignorance, and civic wrongdoing could be overcome.

Notwithstanding advances, there yet remain problems to be solved and differences of opinion to be adjusted before it can be said that our ideas of fitting sanitary standards have been crystallized or brought into unison.

Probably two of the most important questions at present under discussion are those concerned with the presence and significance of streptococci and of "pus cells" in milk-supplies. Thus, according to the standards raised by one authority or another, a milk is adjudged good or bad. In this confusion of what should be considered a proper standard lie the difficulties and differences of opinion. To the writer it seems that the time is now ripe for a full and free discussion of the subject, and that a careful inquiry should be instituted to consider the problem in all its phases and to advise regarding the establishment of suitable standards.

The scope of this paper will be limited to a brief presentation and discussion of these topics, with criticisms and suggestions.

It is now generally agreed that it is a rare thing to be able to draw sterile milk in any moderate quantity from the udders of cows, even while using the greatest of precautions against contamination from the outside; witness the researches of Ward,[2] Reed and Ward,[3] Boekhout and de Vries,[4] von Freudenreich,[5] Conn,[6] Harrison and Cumming,[7] and many others. Such being the case, it is important

to know of what species are those bacteria which inhabit the milk ducts and teat-canals.

These may be divided into two main groups; first, those producing lactic acid fermentation, and second, those producing none. Of the former there exist two types, one giving rise during the fermentation to gas, commonly spoken of as the "*B. aerogenes* type;" the other giving rise to no gas and spoken of as the "*B. lactis acidi* type." Of the latter, i. e., the non-acid group, there exists a variety of forms that are apparently concerned in the causation of odors, flavors, etc., and which at times may even give rise to putrefactive changes. By far the most common, however, of all those bacteria are the lactic acid forms which in market milk, according to many authorities, run from 50 to 100 per cent, and in milk drawn from the udders, according to Harrison and Cumming, 95 per cent.[7]

Concerning the constitution of these acid-forming types, it is generally agreed that to the "aerogenes" type belong *B. aerogenes* (Escherich) for the most part, and in less degree *B. coli;* but all investigators are not of one mind concerning the identity of the bacteria entering into the type "*B. lactis acidi*" (Leichmann).[8] The literature, for example, contains descriptions of organisms under such names as *B. acidi lactici*, *B. lactis acidi*, *Mic. acidi lactici*, *Streptococcus acidi lactici*, *Streptococcus lacticus*, etc., all concerned in the production of lactic acid and coagulation of casein.

A careful consideration of the descriptions of these several bacteria, as given in the literature, leads one to believe that the points of difference lie largely in relatively insignificant variations induced by the non-uniformity of media, or of technique, or in questions of interpretation of results.

The question of the probable unity of the varieties described was first prominently brought forward by Kruse[9] of Bonn in 1903, who seriously doubted the bacillary nature of the lactic acid bacteria of the "*B. lactis acidi*" type, declaring the same to be streptococci closely related on the one hand to *Strept. pyogenes*, and on the other to *Mic. lanceolatus*. His paper, however, was not accompanied by any experimental data, although it must be inferred that he had conducted experiments leading him to this belief. He assigned a secondary rôle to *B. aerogenes* and *B. coli* as lactic acid formers in naturally soured milk.

In the following year there appeared from his laboratory a contribution on this subject by Hölling,[10] containing much experimental evidence of a convincing nature, wherein it is shown that streptococci are in reality the ordinary and most frequent lactic acid formers, and that *B. lactis acidi* and others of that ilk are not bacilli, but streptococci. With the exception of *B. aerogenes* and *B. coli*, this statement covers all previously described organisms recognized by various authorities as being those concerned in the primary lactic acid fermentation of fresh milk.

In ignorance of the papers of Kruse and Hölling, Heinemann,[11] working in the University of Chicago, in 1905, carried out examinations upon milk obtained from the udders of 42 cows under the best of precautions against contamination from the outside, and likewise upon samples of market milk in Chicago, with the object of ascertaining the nature of the bacteria concerned in the souring of milk, together with an inquiry into the status of "*B. lactis acidi.*" For this latter part of the work pure cultures of this bacterium were obtained from several laboratories in this country, and also a series of "starters" such as are sold to dairymen for the ripening of cream. In all these investigations he found that there was but one organism concerned, and that organism was not a bacillus but a streptococcus, and, that in milk naturally soured, forms corresponding to *B. aerogenes* and *B. coli* were numerically less frequent. In this work he confirmed the remarks of Kruse[9] and the experimental data and conclusions of Hölling.[10] Further, he succeeded in isolating from separator slime, cows' feces, and in washing from the cows' udders and teats lactic acid bacteria corresponding in all particulars with the foregoing streptococcus type.

It is not to be inferred that, previous to these investigations, observers had not recorded the occurrence of streptococci in milk. The literature, which need not here be quoted, teems with records of such. But what seems strange is the fact that none regarded his results as other than an expression of a pathological state of the cow's udder, whilst "*B. lactis acidi,*" regarded as a normal inhabitant, was passed by unnoticed as being of the same species, largely it would seem upon the ground of morphology, the bacterium occurring in pairs or short chains of slightly elongated individuals, an expression

of active binary division often seen in cultures of pathogenic streptococci.

In so far as morphological and cultural characters were concerned, Heinemann[11] found, on comparing a series of streptococci from pathological sources, milk, pure cultures of "*B. lactis acidi*," "starters," feces, etc., that there was perfect accord; in all, to be sure, there occurred from time to time variations of a parallel nature in both conditions.

Now, if we regard "*B. lactis acidi*" and *Strept. lacticus*, as Kruse and Hölling call it, to be identical (as I believe they are), in what light, then, are we to regard the presence of streptococci in milk as being an index of disease in the cow? It cannot be denied that cows suffer from inflammation of the udder at one time or another during lactation, and that these lesions are largely caused by the ordinary pyogenic cocci, less frequently by *B. coli* or *B. aerogenes*. Of the cocci it would seem that a streptococcus is the more prominent factor, judging from the researches of Steiger,[12] Bergey,[13] and others, particularly in that form of mastitis known as acute contagious mastitis.

But were we able to distinguish safely the several races of streptococci, it would then be a relatively simple matter to frame standards whereby we might be able to say that a given sample of milk was fit for consumption, whilst another was not, on the grounds that each contained harmless and hurtful cocci, respectively. In this matter we are yet without a test that will permit of assuming a dictatorial attitude. Culturally, no peculiarities of a sufficiently stable nature are apparent whereby any one race of streptococci may be sharply marked off from another. Gordon[14] has proposed the use of such fermentable substances as saccharose, inulin, raffinose, mannit, salicin, coniferin, etc. By their use he believes that he is able to say that this coccus is of salivary origin, that of fecal or pustular; in this way milk streptococci are separated from salivary races by their ability to ferment salicin. On this account Savage[15] regards this test as being of great value in identifying streptococci of bovine origin, but as there occur at times exceptions to this rule, the test, like many of a similar nature already known to us, can then be considered as a presumptive test only, if even that.

Other tests have been proposed and tried out, notably the hemo-

lytic, introduced by Schottmüller,[16] but have led to discordant results. Baumann[17] found that, with a series of pathogenic and non-pathogenic streptococci, the former were uniformly and markedly hemolytic, whereas the latter possessed no hemolytic power; amongst the non-hemolysers he classed the milk streptococci. Müller,[18] comparing the milk streptococci with those from pathological sources, noted that hemolysis occurred amongst both types, and regarded the test as one showing no differences of a sufficiently important nature. He quoted also from the literature in support of this view.

No better also are the tests in the field of agglutination. It is generally conceded that among pathogenic forms no reliable deductions as to possible identity of races can be drawn. Bergey[13] tested the method with a large number of races of milk streptococci using sera prepared from pathogenic varieties, but was unable to come to any satisfactory conclusions. Müller[18] obtained both positive and negative results among a series of pathogenic streptococci tested with pathogenic (homologous) sera, and in lesser degree among races of milk streptococci tested with the same sera. He concluded, however, that a positive result obtained with milk streptococci speaks strongly for the race being pathogenic, but a negative result leaves the question an open one. He is of the opinion that among the milk streptococci are some which are closely related to the pathogenic varieties. Baumann[17] and Savage[15] lay no stress upon agglutination as a means of differentiation, on account of its unreliability.

Tests of pathogenicity, too, are by no means a safe guide in determining the virulence of the races isolated. Beck[19] recorded that not a few of the streptococci isolated by him from the market milk of Berlin were possessed of considerable lethal power toward rabbits and guinea-pigs. Escherich, too, determined that certain races were pathogenic for white mice. But it is a well-known fact that not infrequently streptococci isolated from a variety of pathological sources in human disease produce most irregular results upon inoculation into laboratory animals, and similarly with milk streptococci we find that Reed and Ward[3], Lammeris and van Harrevelt,[20] Brüning,[21] Seiffert,[22] and Bergey[23] (1901) record the statements that the organisms isolated by them were quite avirulent to their laboratory animals, chiefly rabbits and guinea-pigs. And here we must

agree with the statement of Rullmann and Trommsdorff,[24] speaking of this phase of the subject, that such a line of work argues nothing for the pathogenicity of these streptococci toward man, and our deductions must be drawn from another line of facts.

Despite, then, our inability to distinguish racial differences among the streptococci, we cannot but feel sure that there are present in the milk from time to time, cocci which undoubtedly have a virulence all their own toward the human species, and it is the belief of those who are competent to express an opinion that these cocci thus causing human infection are those giving rise to the acute contagious variety of mastitis, or *gelber Galt* of the Germans. Rullmann and Trommsdorff, however, are of the opinion that the presence of streptococci in freshly drawn milk, especially if accompanied with leucocytes, is a sign of a chronic mastitis and the milk is to be regarded with suspicion. In this they agree with the previous statements of Bergey.

The type of disease in human beings occasioned by an infection by such milk takes on two forms, the one marked by distinct and severe gastro-intestinal disturbances combined with general depression and malaise; the other form manifesting itself in a severe faucial angina, tonsilitis, a swelling or suppuration of the submaxillary or cervical glands, occasionally cellulitis, and well-defined constitutional disturbances. Such infections usually take the form of local epidemics confined to the region supplied by the infected milk. Petruschky and Kriebel[25] are of the opinion that the streptococci are responsible for much of the mortality in that form of disease known as summer diarrhea of infants. Examples of infection supposedly caused by these streptococci are given by Holst,[26] Stokes and Wegefarth,[27] Kenwood,[28] and Savage.[29] The latter states that, in an outbreak of sore throat in Colchester, he discovered a cow in the herd of the suspected dairy affected with acute mastitis, and upon removal of the sick animal the epidemic subsided; the milk of the animal contained enormous numbers of streptococci and pus cells. Under such circumstances, then, the discovery of the cause rests, not on bacteriological grounds alone, but most largely upon a clinical examination backed up by bacteriological and microscopical findings.

To face now the question of the importance of the presence of

streptococci in milk, we must acknowledge the correctness of the view that there exists in the udders of practically all normal cows a certain species of bacterium, in numbers greatly exceeding others, and that this bacterium performs a kindly service to the dairyman and is regarded as inoffensive. Then, too, we have to accept the facts recorded by many sanitarians that there are to be observed in the milks of different countries streptococci varying in quantity from 50 to 76.6 per cent of all bacteria present. But have we to subscribe to the interpretations so often attached to the finding of these bacteria, viz., that it is evidence that the cows giving the milk are diseased and that the milk is in consequence unfit for the use of the human subject? I think not. For if we did, then we would be bound to acknowledge that for the greater part milch cows suffer more or less continuously from inflammation of the udder, which is the inference drawn from reading the experiments of Bergey,[13] and Rullmann and Trommsdorff.[24] Kaiser,[30] and Savage,[15] on the other hand, both of whom have gone into examinations of milk and found streptococci to be present in most samples, have not cared to express an opinion in this matter.

The solution of the difficulty seems to me to be furthered if we choose to accept the views of Kruse,[9] Hölling,[10] and Heinemann,[11] namely, that the normal lactic acid bacteria of the udder are not bacilli, as most investigators have thought, but streptococci, and streptococci which, under the conditions in which we find them, are, as has been shown, for the most part non-pathogenic. Then, too, as to their origin, we might indulge in some profitable speculation. Steiger[12] states that there are three routes whereby an infection of the udder may take place: (1) direct infection through the teat-canals, constituting the galactogenous infection; (2) infection through wounds of the udder or teats, on the outside, by way of the lymphatic vessels, the lymphogenous infection; (3) the hematogenous infection, occurring in the course of a general infection having a local starting-point in some distant part of the body. Whatever, then, the mode of infection and the degree of infection produced (leaving aside the question of the acute contagious mastitis), might it not be assumed that after a time the cocci gradually part with their pathogenic powers, and, undergoing some modification, give themselves over to a sapro-

phytic existence, comparable to that led by bacteria in the mouths and intestines of the human subject? Again, it may not be necessary to imagine that an infection takes place according to any of the foregoing ways. It can as readily be conceived that, by a simple fouling of the teats in a "leaky" cow by dung or other dirt, growth of already saprophytic streptococci is permitted to occur along the teat-canal into the udder; or, in a non-"leaky" animal, by a similar style of infection directly after milking, when there yet remains within the orifices of the canals a sufficiency of milk to afford the proper conditions of growth.

That the foregoing arguments for the acceptance of the view that streptococci in milk have not the significance usually attached to their presence will satisfy or cover all points of criticism, I do not pretend to assume. The stand taken is simply put forward in the endeavor to bring into greater harmony facts that appear to be unnecessarily at variance and the cause of so much doubt, as well as of doubtful or seemingly divergent interpretations. The importance of the theme is not insignificant, and if the criticism engendered by this paper will lead to a careful reinvestigation of our facts, we may arrive, it is to be hoped, at no distant day to a much clearer point of view than is at present held regarding the status of the presence of streptococci in milk.

There yet remains, however, another question closely linked up with the preceding, and one, too, that on account of much divergence of opinion requires almost as great attention at the hands of investigators, and that is the presence of the so-called "pus cells," or, more properly, leucocytes in milk.

Too much has been made of this question upon what appears to me to be rather shallow grounds; and, in addition, the somewhat unfortunate choice of the term "pus cell," instead of leucocyte, is to be regretted. As Savage[15] remarks, "That milk should not contain pus cells few will deny, but what constitutes pus in milk? All milk contains leucocytes. When does a leucocyte become a pus cell, and what distinguishes one from the other?" Such is a well-merited challenge to the propriety of the use of the word "pus," as applied to the presence of white blood cells in milk. A decided expression of opinion upon this point is urgently needed, for it must be recognized

that the presence of leucocytes in milk up to a certain point is largely a physiological circumstance; beyond that, a pathological one. What are the bounds to be set in recognizing either of these conditions? Frankly, we do not possess sufficiently reliable data upon which to dogmatize. Bergey,[13] Doane,[31] Savage, and Rullmann and Trommsdorff[24] have demonstrated that in apparently healthy cattle cell numbers may vary among different cows, and in the four mammary quadrants of the udders. To what this variation is due is hard to say. Bergey, and Rullmann and Trommsdorff find that there is a proportional relationship between leucocytes and streptococci, a high leucocyte count usually being accompanied by a correspondingly high streptococcus count; but this state of affairs was not always found to be uniformly so. Savage, on the contrary, could not find any relationship whatever. Whether, apart from any signs of inflammation, chemiotactic substances play any part in leucocytosis due to the residence of saprophytic streptococci within the udder is not known; perhaps they do.

It is interesting to trace the attempts to put this question of leucocytosis upon a practical working basis. Apparently Stokes and Wegefarth[7] in 1897 were the first to propose a plan for the estimation of leucocytes in milk, and with some modification their system is in vogue today in many laboratories in this country. Briefly it was as follows: Ten c.c. of a well-shaken sample of milk were measured out and centrifugalized in a hand centrifuge for five minutes, the supernatant fluid poured off, and a platinum loopful of the sediment spread over a slide, fixed by heat, cleared by ether, and stained with methylene blue and examined under an oil-immersion lens; the number of leucocytes were counted in 10 fields of the microscope, and if they ran over five cells per field, the milk was to be adjudged to contain pus. In 1899 Eastes[31] reported in an indefinite manner upon the presence of pus in milk. Bergey[13] in 1904, using the Stokes-Wegefarth method, decided that if the number of cells were greater than 10 per field, it was indicative of the presence of pus. Stewart[33] used the sediment from 1 c.c. of milk and set the limit of cells at 22 per field. Slack[34], using 2 c.c., spread the sediment over 4 sq.cm. of the surface of a slide, and allowed 50 cells per field of the oil-immersion lens as indicative of pus formation.

Writing in 1905 under the title, "The Doane-Buckley Method of Determining Leucocytes," Doane[31] criticizes the foregoing methods in these words: "There is but one element of scientific accuracy in the whole process. . . . the using of a definite quantity of milk"—which criticism seems to be largely justified. The later modifications differ only in the attempts to have a more or less definite area of the slide covered with sediment, which in fact adds little to the accuracy of the technique. Doane describes the technique of the Doane-Buckley method as follows: 10 c.c. of milk are centrifuged for four minutes in a graduated centrifuge tube at an approximate speed of 2,000 revolutions per minute; the cream is then lifted out with a cotton swab and centrifuged for another minute; the cream is again taken out as

before, and, without disturbing the sediment, the overlying milk is syphoned off, leaving fluid to the depth of $\frac{1}{8}$-inch above the sediment; two drops of a saturated alcoholic solution of methylene blue are now added, mixed thoroughly with the sediment, and set aside in boiling water for two or three minutes to allow the leucocytes to take up the stain; hot water is then poured in to fill up to the 1 c.c. mark on the graduated tube; the contents are now shaken vigorously and a portion taken up in a capillary pipette and placed upon the ruled surface of a Thoma-Zeiss blood counter and covered with the cover-glass; allowing a minute for the cells to settle down, a total count of all the squares is made, or, if the cells are very many, then an average of the whole number may be obtained by the counting of say 10 or 15 squares. Calculation of the cells per c.c. of the milk is then made. The fluid over the total squares represents 0.1 c.mm., or 0.0001 c.c. Then say that there were 50 cells counted in the whole ruled part of the slide, which is equivalent to 500,000 cells, but as this number came from 10 c.c. of milk, 1 c.c. contains 50,000 leucocytes. This method certainly is free of any criticism upon the grounds of lack of scientific accuracy, as it is plainly that method used by clinicians in the enumeration of the cells of the blood with slight modifications to suit the conditions.

Ward[35] upholds the accuracy of the Doane-Buckley method in an article published this year, stating that it gives much more satisfactory results than that of Stewart, the count running from 4 to 40 times higher.

Quite independently of Doane and Buckley, Savage[15] this year has practically worked out the same method, varying the details in some degree. He, too, felt the burden of the inaccuracy of the Stokes-Wegefarth plan and so set about to develop one more suitable and more scientifically precise. As carried out by Savage, the process consists in taking 1 c.c. of the sample and putting it into a 20 c.c. graduated centrifuge tube, filling up to the 20 c.c. mark with Toisson's solution; after mixing well, the sample is centrifuged for 10 minutes, the cream is broken up thoroughly with a glass rod, and again whirled for 10 minutes. All the fluid is now removed but the last 1 c.c., which is stirred up well and the required amount is placed upon a Thoma-Zeiss counter and counted, the calculation of the cells being done by means of a special formula and rendered in cubic millimeters, thus making the counting part of the method apparently unnecessarily difficult, although its accuracy cannot be called into question.

Another system developed in Germany by Trommsdorff[36] and published this year differs in all respects from any of the foregoing, and although it is more accurate than the Stokes-Wegefarth method, it is yet only capable of furnishing qualitative results, and not quantitative as should be the case, such as is afforded by Doane and Buckley's and by Savage's methods. In this method 5 c.c. of milk are taken and put in specially devised tubes, which are drawn out to capillary points and there carefully etched into divisions of 0.001 c.c., running from 0.001 to 0.02 c.c. in volume. Upon being centrifuged for a few minutes, the amount of sediment is read off and the amount calculated in volumes per 1,000 or 10,000 as desired. It can readily be seen that these results, qualitative as they are, may be invalidated by the presence of foreign matter blocking up the capillary tube, although the inventor claims that under his care the instrument is quite free from this objection. Its usefulness may possibly be further impaired by the accumulation of other material in the milk than pus, e.g., fibrin and mucin.

Of these several methods devised for the recognition of pus in milk only three merit any consideration, namely, those of Doane and Buckley, of Savage, and of Trommsdorff. In point of accuracy and true analysis, the first two methods and the last stand in parallel contrast with each other, exactly as do the two methods for the estimation of leucocytes in the blood, i. e., by actual count and by the hematokrit; and of them, the one freer from error and more desirable is that by actual count.

To go into the results obtained by the authors would be to lengthen this paper very considerably. Suffice it to say that each finds much variation in the leucocyte count among individual cows, between the four mammary quadrants of each cow, and between individual cows' milk and that of the whole herd. As regards the question of standards, Rullmann and Trommsdorff consider that any sample yielding over 1 volume per mille is evidence enough that there is pus in the milk. To quote Savage,[15] "I cannot differentiate between a leucocyte and a pus cell, and I am not prepared at this stage to lay down an arbitrary standard as to what number of leucocytes per cb.mm. is to be designated *pus* in the milk." He also affirms that there is no relation between leucocytes and streptococci, so far as his figures go. Doane,[31] on the other hand, is more specific and states that a milk containing 500,000 cells per c.c., together with the presence of fibrin, is to be regarded as suspicious, while a content of 1,000,000 per c.c. associated with fibrin is conclusive of the presence of pus, i. e., evidence of mastitis.

This, then, closes the presentation of a subject that very evidently is at present in a rather chaotic state, but one that invites our further careful scrutiny, deliberation, and judgment, before it can be said that the milk problem is a settled one.

The following conclusions are offered:

1. It seems imperative to secure an early adjustment of the divergence of views of the taxonomic status of the so-called *B. lactis acidi.*

2. The statements of Kruse, Hölling, and Heinemann cast considerable doubt upon the value heretofore entertained regarding the significance of streptococci in milk.

3. It is not excluded by the evidence that pathogenic streptococci are to be found at times in milk; in fact, recorded observations make

this certain, the contamination arising from clinically recognizable cases of mastitis in the herds.

4. We are not as yet in possession of any reliable method for distinguishing a non-pathogenic from a pathogenic streptococcus.

5. The sanitary significance of the so-called "pus cell" has been greatly overrated. More scientific attention should be given to the study of the phenomena of lactic leucocytosis, together with a more accurate method of enumeration, such as that of Doane and Buckley, or of Savage.

6. Particularly, should more attention be given to veterinary inspection of the cows' udders, with less absolute dependence upon laboratory examination of milk for signs of infectious processes.

7. The time seems ripe for throwing open the whole question for discussion, and the framing of new rules to cover points raised and accepted.

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